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Artículo Original

MOLECULAR DATA SUGGEST THAT *Calomys boliviae* (THOMAS, 1901) WOULD BE A VALID SPECIES BELONGING TO THE WESTERN LINEAGE OF THE *Calomys callosus* COMPLEX (RODENTIA, CRICETIDAE)

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ABSTRACT. The *Calomys callosus* complex involves species with medium to large body sizes for the genus and so far includes *C. callosus* sensu stricto, *C. fecundus*, *C. callidus*, *C. venustus*, *C. tocantinsi,* and *Calomys* sp., the latter representing populations from the Beni savannah of Bolivia. There is also a species named *C. boliviae* from the Yungas ecoregion of central Bolivia, which has often been synonymized with *C. fecundus*, a species found in the Tucumane-Bolivian Yungas ecoregion of northern Argentina and southern Bolivia. In the present study, we report the molecular analysis, based on the mitochondrial Cyt-b gene, of specimens of *Calomys* sampled in the Bolivian Yungas in an area close to the type locality of *C. boliviae*. Phylogenetic results showed that the sequences of the specimens studied formed a clade distinct from *C. fecundus* and that, taking into account its geographical closeness to the typical area of *C. boliviae*, they could be representing this nominal form. If this hypothesis is true, *C. boliviae* should be resurrected as a valid species of *Calomys* belonging to the western lineage within the *C. callosus* complex.

RESUMEN. LOS DATOS MOLECULARES SUGIEREN QUE *Calomys boliviae* (THOMAS, 1901) SERÍA UNA ESPECIE VÁLIDA PERTENECIENTE AL LINAJE OCCIDENTAL DEL COMPLEJO *Calomys callosus* (RODENTIA, CRICETIDAE). El complejo *Calomys callosus* comprende especies con tamaños corporales medianos a grandes para el género, y hasta ahora incluye *C. callosus* sensu stricto, *C. fecundus*, *C. callidus*, *C. venustus*, *C. tocantinsi* y *Calomys* sp., esta última corresponde a poblaciones de la sabana del Beni de Bolivia. También hay una especie denominada *C. boliviae* de la ecorregión Yungas del centro de Bolivia, que, a menudo, ha sido sinonimizada con *C. fecundus*, una especie que se encuentra en la ecorregión de las Yungas Tucumano-bolivianas del norte de Argentina y el sur de Bolivia. En el presente estudio informamos el análisis molecular, basado en el gen mitocondrial *Cyt-b*, de especímenes de *Calomys* muestreados en las Yungas bolivianas en un área cercana a la localidad tipo de *C. boliviae*. Los resultados filogenéticos mostraron que las secuencias de los especímenes estudiados formaron un

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clado distinto de *C. fecundus* y que, teniendo en cuenta la cercanía geográfica del área típica de *C. boliviae*, podrían estar representando a esta forma nominal. Si esta hipótesis resulta ser cierta, *C. boliviae* debería ser resucitada como una especie válida de *Calomys*, perteneciente al linaje occidental dentro del complejo *C. callosus*.

Key words: Cytochrome b, phylogeny, rodents, Sigmodontinae, Bolivian Yungas.

Palabras clave: Citocromo b, filogenia, roedores, Sigmodontinae, Yungas bolivianas.

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INTRODUCTION

Calomys Waterhouse, 1837 (Sigmodontinae, Cricetidae, Rodentia) is a very diverse genus within the Phyllotini tribe (D'Elía & Pardiñas 2015). The number of recognized species varies between 12 and 20 (Almeida et al. 2007; Haag et al. 2007; Salazar-Bravo 2015, 2017), ranging from Venezuela to southern Patagonia in Argentina. It is composed of several small-bodied species (< 20 grams of mass) mainly distributed in the lowlands of eastern tropical and temperate South America (e.g., *C. laucha*, *C. tener*, among others), and medium-large forms (ranging from 30 to 50 grams), including lowland species of eastern Brazil such as *C. cerqueirai* Bonvicino, Oliveira & Gentile, 2010, *C. expulsus* (Lund, 1841), and the members of the *C. callosus* complex, with a wider distribution. This complex includes at least five nominal species, namely: *C. callosus* (Rengger, 1830) sensu stricto (s.s., thereafter), *C. venustus* (Thomas, 1894), *C. callidus* (Thomas, 1916), *C. fecundus* (Thomas, 1926), *C. tocantinsi* Bonvicino, Lima & Almeida, 2003, and an as yet unnamed sixth form, *Calomys* sp., from the Beni savannah of northern Bolivia, which is worthy of a formal description in the future (Salazar-Bravo et al. 2002; Almeida et al. 2007; Haag et al. 2007; Bonvicino et al. 2010; González-Ittig et al. 2022).

In Bolivia, there is also a medium-bodied form, *Calomys boliviae* (Thomas 1901), which has hardly been studied since its original description. This species has often been treated as a junior synonym of *C. fecundus*, which effectively belongs to the *C. callosus* complex (González-Ittig et al. 2022). However, this synonymy was made without a direct comparison between them, and it is not clear if they are the same species and if *C. boliviae* is a member of the *C. callosus* complex or not (Pinotti et al. 2020). According to Thomas (1926), the type locality of *C. fecundus* is Tablada, 2000 m, Department of Tarija, Bolivia, while that of *C. boliviae*, mentioned by Thomas (1901), is Solacama River (incorrectly written as Solocame River in the original description), 1200 m, Department of La Paz, Bolivia. Oldfield Thomas in 1901 also gave the geographical coordinates 67° W, 16° S, which are indicative of the area but do not correspond exactly to the mentioned river (**Fig**. 1). Also in the original descriptions, morphological measurements of *C. fecundus* were reported to be larger than those of *C. boliviae*. However, Thomas (1901, 1926) did not explicitly compare the two species. Olds (1988) noted that adult females of *C. boliviae* had five pairs of mammae, while those of *C. fecundus* had seven pairs. From a different perspective, Pinotti et al. (2020) used ecological niche modeling to show that none of the potential distribution models predicted the presence of *C. fecundus* in the type locality of *C. boliviae*. Therefore, the authors proposed that *C. fecundus* would be restricted to the Tucumane-Bolivian Yungas, while *C. boliviae* would be restricted to the northern Bolivian Yungas. It should be noted that these ecoregions are climatically and biogeographically distinct and are separated by the Bolivian Montane Dry Forest, which could be a barrier to gene flow between the northern and southern populations of medium-large-sized *Calomys*. The northern Bolivian Yungas are more humid and less seasonal and benefit from the moisture brought by the trade winds. In contrast, the Tucumane-Bolivian Yungas suffer more directly from southerly cold fronts (Ibisch & Mérida 2004). *Calomys fecundus* has been collected in Argentina in the provinces of Jujuy, Salta, Tucumán, and Santiago del Estero, and in Bolivia in the departments of Tarija and Chuquisaca (Salazar-Bravo et al. 2002; Salazar-Bravo 2015). At the chromosomal level, specimens of *C. fecundus* from Famaillá, in the province of Tucumán, present a 2n = 54 (FN = 66; Hurtado de Catalfo & Wainberg 1974), and the same 2n has been

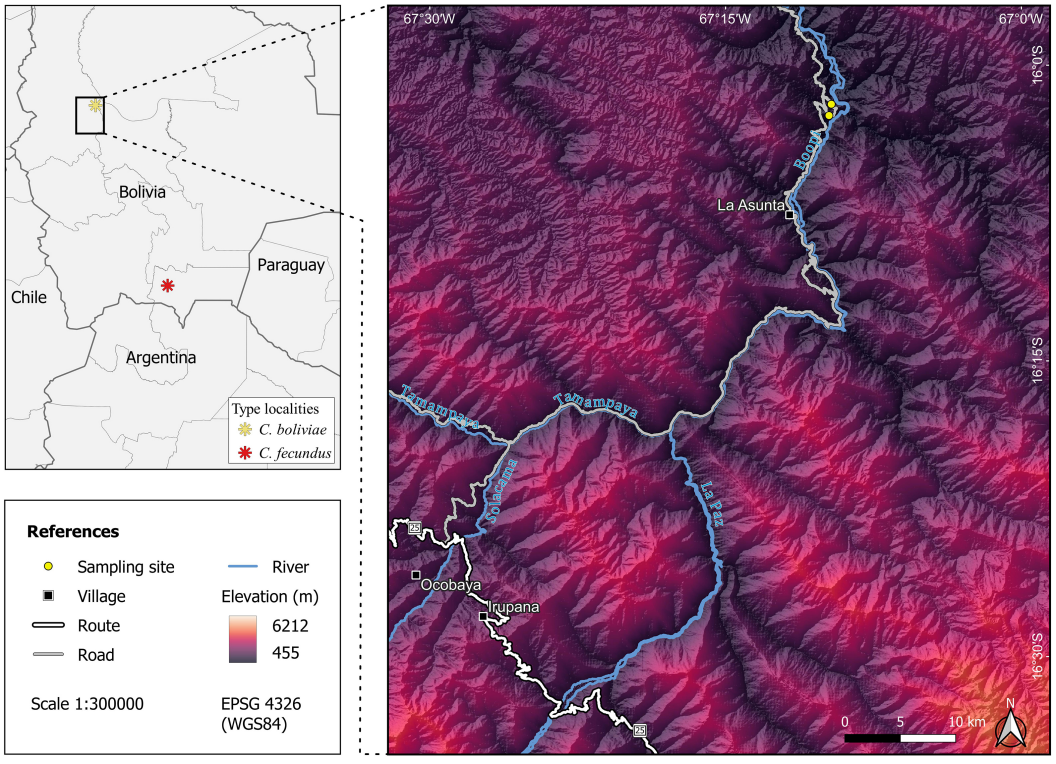

Fig. 1. Upper left panel: map showing the type localities of *Calomys boliviae* (yellow star: Río Solacama, department of La Paz) and *Calomys fecundus* (red star: Tablada, department of Tarija) in Bolivia. The right panel shows a zoom of the area where the type locality of *C. boliviae* would be found. The sampling sites of the three individuals of *C. boliviae* collected in this study are marked with yellow dots near the community of San José, municipality of La Asunta, province of Sud Yungas, department of La Paz. The map includes the area with the coordinates 67° W 16° S, given by Thomas (1901). The Solacama River is highlighted and localities close to the river, such as Ocobaya and Irupana, are also shown.

reported for animals from Tarija and Chuquisaca in southern Bolivia (Salazar-Bravo et al. 2002). There has even been a phylogeographic study for *C. fecundus* (Pinotti et al. 2020), so it is a well-known species. In contrast, *C. boliviae* is known only from its type locality and from other nearby localities, which were mentioned by Thomas (1901) on page 253, with little precision, as follows "*Other specimens from Yungas, 1500 m, and Astillero, 2700 m.*" In total, five specimens were originally examined, all collected by Perry O. Simons. Up to today, *C. boliviae* has not been assessed at the molecular or cytogenetic level.

In 2019, the Bolivian Ministry of Health reported a cluster of 9 cases of hemorrhagic fever (4 of which were fatal) linked to the municipalities of Caranavi and Guanay, both in the Department of La Paz. The study by Loayza Mafayle et al. (2022) described that the Chapare mammarenavirus was the etiological agent of the Chapare hemorrhagic fever and that it was associated with the rodent *Oligoryzomys microtis* (J. A. Allen, 1916). During that investigation, some of the infected patients were unsure of the site of infection, reporting that it may have occurred near the community of San José, municipality of La Asunta, province of Sud Yungas, Department of La Paz, Bolivia. As a result, several captures were performed in this area, which is relatively close to the type locality of *Calomys boliviae* (**Fig**. 1). In the present study, we used the mitochondrial DNA cytochrome b gene (Cyt-b) to identify individuals of *Calomys* sampled in the Bolivian Yungas ecoregion. We performed phylogenetic analyses and discussed the taxonomic status of these specimens and their relationship to the *C. callosus* complex.

MATERIAL AND METHODS

The sampling was performed in the context of an outbreak of the Chapare hemorrhagic fever disease

caused by a mammarenavirus. Small rodents were captured using live traps baited with a mixture of oats, vanilla extract, and peanut butter. Traps were placed in the Bolivian Yungas forests (**Fig**. 1), but at the edge of a human-modified area, in a peridomestic sector with coca and banana plantations. Eighteen lines of 25 traps each were set for four consecutive nights (total effort of 1800 trap nights).

All captured animals were killed by cervical dislocation under isoflurane anesthesia. Blood samples were taken by periorbital extraction and were conserved in FTA Cards. For most specimens, the brains were extracted for virological testing according to Loayza Mafayle et al. (2022).

A general external and qualitative review of some cranial and dental structures of the collected specimens was performed. The nomenclature used to describe the anatomy of the cranium and mandibles was according to Hershkovitz (1962) and Reig (1977). All the studied specimens were deposited in the Colección Boliviana de Fauna (acronym = CBF), Museo Nacional de Historia Natural, Universidad Mayor de San Andrés, La Paz, Bolivia.

DNA extraction, PCR amplifications, and sequencing

For this study, only specimens identified as *Calomys* were examined at the molecular level. Total genomic DNA was extracted from the FTA Cards using the DNeasy Blood & Tissue kit from Qiagen in a biosafety level 2 laboratory. Proteinase K and RNAse were used in the extraction procedure to inactivate potential virus particles (*Orthohantavirus* and/or *Mammarenavirus*) present in the samples. The mtDNA cytochrome b (Cyt-b) gene was amplified with the primer combination *Mus14095* and *Mus15398* according to the conditions described in González-Ittig et al. (2010); the same primers were used for sequencing.

Amplification products were purified using the QIAquick gel extraction kit (Qiagen Inc., Germany) according to the recommendations of the manufacturer and sequenced with a 3500 Avant genetic analyzer (Applied Biosystems).

For phylogenetic studies, we included sequences from species of the *Calomys callosus* species complex: Large vesper mouse (*C. callosus* s.s.), Fecund vesper mouse (*C. fecundus*), Tocantins vesper mouse (*C. tocantinsi*), Córdoba laucha (*C. venustus*), Crafty vesper mouse (*C. callidus*), and *Calomys* sp. from the Beni savannah in Bolivia (**Table** 1). We also included sequences of the Caatinga vesper mouse (*Calomys expulsus*) and the Cerqueira's vesper mouse (*C. cerqueirai*) and, as outgroups, the Drylands vesper mouse [*C. musculinus* (Thomas, 1913)] and the Small vesper mouse [*C. laucha* (G. Fischer, 1814)]. Nucleotide alignments were obtained using MAFFT version 7 (Katoh & Standley 2013), using default parameters.

Phylogenetic analyses

Maximum parsimony (MP) analysis was performed with Paup 4.0.b10 (Swofford 1998); characters were unordered and equally weighted. Nonparametric bootstrap support values were calculated based on 1000 replicates. The best-fitting model of sequence evolution was selected using jModeltest 2 (Darriba et al. 2012) in which the likelihood scores for 88 different models were computed. The HKY+G model was selected using the Bayesian information criterion and the following starting parameters were subsequently used in the Bayesian inference (BI) analyses: a base frequency of A = 0.3218, C = 0.2654, G = 0.1102, T = 0.3026; a transition/transversion ratio = 4.9159; and a gamma distribution = 0.1910. The BI analyses were performed using MrBayes version 3.2 (Ronquist et al. 2012) with two independent Markov chain Monte Carlo (MCMC) runs, each with one cold and three heated chains. Runs were performed for 10 million generations, and trees were sampled every 1000 generations. We discarded the first 25% of the samples as "burn-in" and the two runs converged on very similar posterior estimates with an average standard deviation of split frequencies of 0.008. We assessed convergence and mixing visually using Tracer version 1.5 to plot likelihood scores for all parameters and by calculating the parameter effective sample sizes (Rambaut & Drummond 2007). ML trees were constructed using the online version of PhyML version 3.0 (http://www.atgc-montpellier.fr/phyml/) (Guindon et al. 2010). The HKY+G+I model was selected using the Akaike information criterion and included the parameters described above plus the proportion of invariable sites = 0.4010 and 1000 bootstrap replicates.

Based on the Cyt-b data, the Kimura 2-parameter (K2P) genetic distances within and among clades were calculated using Paup 4.0.b10.

RESULTS AND DISCUSSION

The overall capture success rate was 2.53 %. The most productive lines were those close to human-intervened environments (peri-domicile, football field, road to telephone antenna). The capture success is consistent with previous studies conducted by Revollo-Cadima et al. (2021) in semi-intervened

Table 1

Basic data of the specimens referred to *C. boliviae* and *C. callosus* complex species used in this study. Voucher numbers, GenBank accession numbers of the Cyt-b gene are indicated. For each sampling, site the respective geographical coordinates are indicated. Arg: Argentina; Bol: Bolivia; Bra: Brazil; Par: Paraguay. For *C. boliviae* only: CBF: Colección Boliviana de Fauna; the field numbers are provided between parentheses.

Species	Voucher numbers	GenBank number	Sample location	Latitude	Longitude
C. boliviae	CBF-11850 (San José-6)	PQ680042	San José community, La Asunta	-16.042522	-67.162569
C. boliviae	CBF-11851 (San José-18)	PQ680043	municipality, province of Sud Yungas,	-16.032947	-67.160619
C. boliviae	CBF-11852 (San José-27)	PQ680044	department of La Paz, Bolivia	-16.032947	-67.160619
Calomys sp.	NK27668	AY033153	El Valle, Beni, Bol.	-13.65	-64.4333333
Calomys sp.	NK37800	AY033154	Villa Olga, Beni, Bol.	-13.6333333	-64.7166667
Calomys sp.	NK37735	AY033155	La República, Beni, Bol.	-13.1666667	-64.1666667
Calomys sp.	NK37739	AY033157	La República, Beni, Bol.	-13.1666667	-64.1666667
Calomys sp.	NK37787	AY033156	Chumano, Beni, Bol.	-13.6166667	-64.8166667
C. fecundus	NK23650	AY033164	Tucumilla, Tarija, Bol.	-21.45	-64.8166667
C. fecundus	NK23354	AY033170	Camatindy, Tarija, Bol.	-21.0166667	-63.3833333
C. fecundus	AK15276	AY033173	Yerba Buena, Tucumán, Arg.	-26.8166667	-65.3666667
C. fecundus	INEVH-35595	MN105639	El Ceibal, Jujuy, Arg.	-24.302	-65.2794
C. fecundus	INEVH-1305	MN105616	Libertador San Martín, Jujuy, Arg.	-23.7939	-64.7608
C. fecundus	INEVH-1306	MN105617	Libertador San Martín, Jujuy, Arg.	-23.7939	-64.7608
C. fecundus	INEVH-36531	MN105633	Yuto, Jujuy, Arg.	-23.6439	-64.5414
C. fecundus	INEVH-36518	MN105632	Yuto, Jujuy, Arg.	-23.6439	-64.5414
C. fecundus	INEVH-37828	MN105643	Famaillá, Tucuman, Arg.	-27.0311	-65.3778
C. fecundus	INEVH-37823	MN105642	Famaillá, Tucuman, Arg.	-27.0311	-65.3778
C. fecundus	INEVH-37988	MN105673	Arraga, Santiago del Estero, Arg.	-28.0264	-64.2409
C. fecundus	INEVH-37966	MN105667	Arraga, Santiago del Estero, Arg.	-28.0264	-64.2409
C. fecundus	Met-9	MN105683	San José de Metán, Salta Arg.	-25.5487	-64.9479
C. fecundus	Pk-7	MN105685	Las Víboras, Salta, Arg.	-25.0149	-64.5947
C. venustus	AK15337	AY033176	15km NE from Villa Ojo de Agua, Santiago del Estero, Arg.	-29.4333333	-63.5666667
C. venustus	ST2	MN946552	Santiago de Temple, Córdoba, Arg.	-31.3833	-63.4167
C. venustus	CE35	MN946553	Capilla de los Remedios, Córdoba, Arg.	-31.480278	-64.01
C. venustus	CE36	MN946554	Capilla de los Remedios, Córdoba, Arg.	-31.480278	-64.01
C. venustus	CE39	MN946555	Capilla de los Remedios, Córdoba, Arg.	-31.480278	-64.01
C. venustus	CE46	MN946556	Capilla de los Remedios, Córdoba, Arg.	-31.480278	-64.01
C. venustus	PY13	KY753959	Papagayos, San Luis, Arg.	-32.675	-64.9863889
C. venustus	INEVH-37606	MN946557	Pozo del Molle, Córdoba, Arg.	-32.0219444	-62.8952778
C. venustus	INEVH-37757	MN946558	Esperanza, Santa Fe, Arg.	-31.4488889	-60.9302778
C. venustus	INEVH-37763	MN946559	San Jorge, Santa Fe, Arg.	-31.8733333	-61.8538889
C. venustus	INEVH-37800	MN946560	Carlos Pellegrini, Santa Fe, Arg.	-32.0502778	-61.78
C. venustus	Mol13	MN946561	Molinari, Cordoba, Arg.	-31.199359	-64.47688
C. venustus	ACO31	MN946562	Reserva La Florida, San Luis, Arg.	-33.126408	-66.027556
C. venustus	Vaq22	MN946563	Reserva Vaquerías, Córdoba, Arg.	-31.1252778	-64.4627778
C. callosus	NK72344	AY033187	Monte Palma, Presidente Hayes, Par.	-22.6	-59.5166667
C. callosus	NK72378	AY033188	Filadelfia, Boquerón, Par.	-22.3333333	-60.0333333
C. callosus	JAO356	DQ447279	Barão de Melgaço, Mato Grosso, Bra.	-16.1938889	-55.9625
C. callosus	LBCE3054	DQ447280	Corumbá, Mato Grosso do Sul, Bra.	-19.0080556	-57.65
C. callosus	LBCE5682	DQ447282	Corumbá, Mato Grosso do Sul, Bra.	-19.0080556	-57.65
C. callosus	INEVH-36092	MN946564	Colonia Buena Vista, Formosa, Arg.	-25.04048	-58.46585
C. callosus	INEVH-36094	MN946565	Colonia Buena Vista, Formosa, Arg.	-25.04048	-58.46585
C. callosus	INEVH-36096	MN946566	Colonia Buena Vista, Formosa, Arg.	-25.04048	-58.46585
C. callosus	INEVH-36100	MN946567	Colonia Buena Vista, Formosa, Arg.	-25.04048	-58.46585
C. callosus	INEVH-36109	MN946568	Colonia Buena Vista, Formosa, Arg.	-25.04048	-58.46585
C. callosus	INEVH-36124	MN946569	Colonia Buena Vista, Formosa, Arg.	-25.04048	-58.46585
C. callosus	CG54	MN946570	Reserva el Bagual, Formosa, Arg.	-26.306143	-58.816791
C. callosus	CH6	MN946571	Ingeniero Juarez, Formosa, Arg.	-23.8466667	-61.8383333
C. callosus	CH8	MN946572	Ingeniero Juarez, Formosa, Arg.	-23.8466667	-61.8383333
C. callosus	CH10	MN946573	Ingeniero Juarez, Formosa, Arg.	-23.8466667	-61.8383333
C. callosus	NK12308	AY033177	Santiago de Chiquitos, Santa Cruz, Bol.	-18.3	-59.6
C. callosus	NK13009	AY033182	San Ramón, Santa Cruz, Bol.	-16.52	-62.47
C. callosus	BYU19015	AY439001	Concepción, Santa Cruz, Bol.	-15.6521	-62.0375
C. tocantinsi	ARB12	DQ447277	Paraña, Tocantins, Bra.	-12.5933333	-47.8611111
C. tocantinsi	ARB55	DQ447278	Paraña, Tocantins, Bra.	-12.5933333	-47.8611111
C. tocantinsi	FSF49r	KR190447	Santana do Araguaia, Pará, Bra.	-9.675	-50.1341667
C. tocantinsi	FSF145r	KR190460	Santana do Araguaia, Pará, Bra.	-9.675	-50.1341667
C. tocantinsi	FLV74r	KR190454	Lagoa da Confusão, Tocantins, Bra.	-10.7805556	-49.6263889
C. callidus	INEVH-32471	MN946574	Victoria, Entre Ríos, Arg.	-32.6411	-60.1711
C. callidus	INEVH-32412	MN946575	Victoria, Entre Ríos, Arg.	-32.6411	-60.1711
C. callidus	INEVH-28104	MN946576	Los Conquistadores, Entre Ríos, Arg.	-30.5675	-58.4522222
C. callidus	INEVH-38723	MN946577	Nogoyá, Entre Ríos, Arg.	-32.3677778	-59.7786111
C. callidus	INEVH-38745	MN946578	Nogoyá, Entre Ríos, Arg.	-32.3677778	-59.7786111

Table 2

External measurements of the 3 specimens referred to *Calomys boliviae* collected in the community of San José, municipality of La Asunta, province of Sud Yungas, department of La Paz, Bolivia.

Voucher numbers	Total length	Head and body length	Tail length	Ear length	Body mass (g)
CBF-11850	143	81	62	15	12
CBF-11851	183	105	78	16	25
CBF-11852	180	106	74	17	34

environments in the north of the Department of La Paz, Bolivia. Rodents and marsupials were captured, but only rodents could be identified to genus level in the field: *Akodon* (n = 1), *Calomys* (n = 3), *Euryoryzomys* (n = 4), *Hylaeamys* (n = 1), *Neacomys* (n = 1), *Nephelomys* (n = 1), *Oligoryzomys* (n = 16), and *Proechimys* (n = 1); the captured marsupials were n = 6. No infected specimens were detected near the community of San José, neither with mammarenaviruses nor with orthohantaviruses.

Regarding the genus *Calomys*, only three specimens were captured and examined; the coordinates of their capture sites are listed in **Table** 1 and marked with yellow dots in **Figure** 1. The external measurements of these specimens are summarized in **Table** 2; two were adults and one was a juvenile. The external morphology of the species of this genus, especially those of medium-large-sized, is remarkably similar, making them difficult to identify in the field (Hershkovitz 1962; Reig 1977). These species are characterized by their short limbs and medium-sized ears (< 20 mm), with generally well-differentiated dorsal and ventral coloration. The dorsal coloration is grayish-brown with a reddish hue on the upper and lateral parts of the body, while the ventral surface ranges from white to grayish-white. They have large eyes and white post-auricular patches that are weakly to moderately defined but visible in most species. They also have small feet (< 23 mm) that are covered with white hair on the dorsal surface except for the heels. The tail is less than or equal to the head and body length, bicolored, thin, well covered with hairs, and lacking a terminal tuft (Salazar-Bravo 2015).

In relation to the morphological characteristics of the specimens captured in the community of San José, the skull has a straight lateral profile, a robust and short rostrum, opisthodont upper incisors with bright orange enamel, and broad nasal bones that are slightly curved downward at the tip, protruding and thus concealing the incisors when viewed from above (**Fig**. 2a); these features are also noticeable in lateral view (**Fig**. 2b). The interorbital region is flat, with sharp and posteriorly divergent frontal margins that produce supraorbital shelves. The coronal suture is broadly "U" shaped, the palate is long and wide, with long incisive foramina extending beyond the anterior roots of the first upper molars, and the mesopterygoid fossa is slightly wider than in other medium-large sized *Calomys* species, with its anterior portion slightly wider than the rest of the fossa (Hershkovitz 1962; Reig 1977; Salazar-Bravo 2015).

For molecular analyses, the Cyt-b sequences of these specimens were aligned with those of other species of the *Calomys callosus* complex; the matrix presented a total of 278 parsimony-informative and 28 uninformative characters; the consistency and retention indices were 0.643 and 0.894, respectively. The general topology of the phylogenetic tree (**Fig**. 3) is consistent with the existence of eastern and western lineages as proposed by Salazar-Bravo et al. (2002) and further developed by González-Ittig et al. (2022). All methods resolved the species of the complex as monophyletic, and relationships were also organized in two main clades. The eastern lineage consists of *C. callidus*, *C. callosus* s.s., and *C. tocantinsi*. The western lineage includes *C. fecundus*, *C. venustus*, *Calomys* sp., and, most importantly, a clade with the San José specimens, which most likely correspond to *C. boliviae*. This assumption is based on the fact that Thomas (1901) originally referred to the collected specimens as *Eligmodontia callosa boliviae* and stated on page 254, that "*This Eligmodontia is clearly the Bolivian representative of E. callosa, Rengg from which it differs by its rather smaller teeth and more buffy under surface*." In the present study, the specimens from San José undoubtedly are part of the *C. callosus* complex and were collected at sites that are very close to the geographical coordinate provided by Thomas (1901) and relatively close to the Solacama River mentioned as the type locality. It is important to highlight that it is difficult to establish a precise site as a type locality, as Thomas

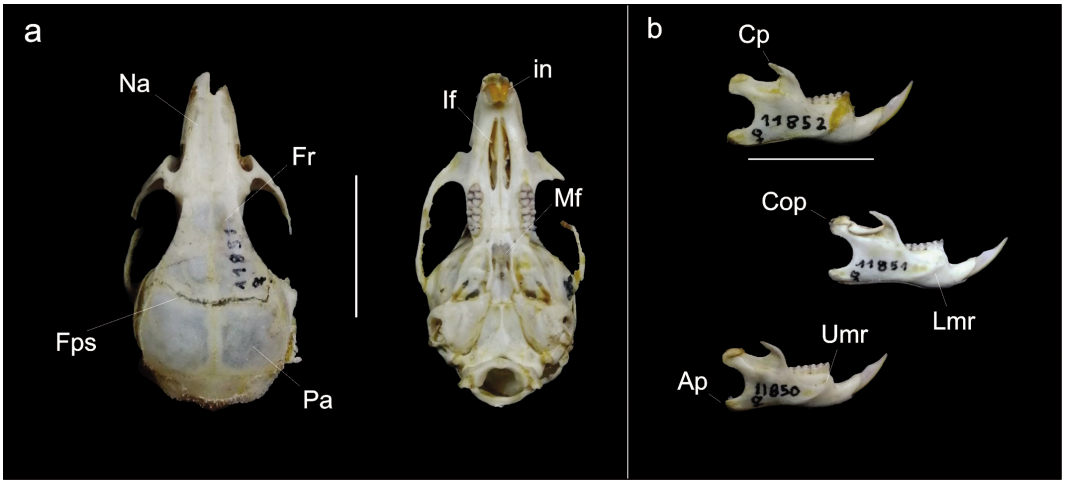

Fig. 2. Cranium and mandibles of specimens referred to *C. boliviae* collected in the Department of La Paz, Bolivia: a) dorsal and ventral views of the cranium of the specimen CBF-11851; b) labial views of the mandibles of three specimens CBF-11850, CBF-11851 and CBF-11852. References: Na: nasal; Fr: frontal; Fps: fronto-parietal suture; Pa: parietal; In: incisors; If: incisive foramen; Mf: mesopterigoid fossa; Cp: coronoid process; Co: condylar process; Lmr: lower masseteric ridge; Umr: upper masseteric ridge; Ap: angular process. Scale bar = 10 mm. Photographs by Luis Andrés Castillo; standardized images by Enzo Rossi.

(1901) did not give details of the sites where Perry O. Simons collected the rodents used for the original description. It is interesting to mention that López Beltrán (2008) compiled and translated the chronicles of the naturalist Luigi Balzan's travels in Bolivia and Paraguay between 1885 and 1893. According to these travel chronicles, there was a road and a suspension bridge over the Solacama River between Ocobaya and Irupana localities. One could therefore conjecture that Simons collected at or near this area years later. **Figure** 1 shows these localities and also the sector that includes the coordinates given by Thomas (1901). It can be seen that the sampling sites of the present study (yellow dots) are near the area where probably Simons collected, which gives us a confident presumption that the Cyt-b clade recovered for the individuals from San José does indeed correspond to *C. boliviae*.

If we consider the K2P genetic distance between the species of the eastern lineage, it can be observed that it ranged from 0.85 to 1.57% (gray, **Table** 3). The genetic distances of the western group ranged from 1.00 to 1.41% (orange, Table 3). The genetic distance between the two main clades ranged from 1.8 to 3.04% (yellow, **Table** 3). Particularly, for *C. boliviae*, the smallest distance to the other species was 1.31% with *C. venustus* and the largest was 2.79% with *C. callidus*. Although in general, these are all low distances, these values plus the obtained topology provide evidence that *C. boliviae* is different from *C. fecundus* and from *Calomys* sp., both of which are also found in Bolivia.

With the results of the present study, we are confident that *C. boliviae* is indeed part of the *C. callosus* complex and, in particular, part of the western lineage, as predicted by González-Ittig et al. (2022). This finding also supports the results of Pinotti et al. (2020), who suggested, based on ecological niche modeling, that *C. fecundus* and *C. boliviae* should be considered different species since modeling of the former did not show its presence at the type locality of the latter.

In relation to health issues, several species of the *C. callosus* complex are of zoonotic importance, reinforcing the interest in having robust taxonomic knowledge for this group of species. Accurate species identification and range mapping provide a better understanding of disease epidemiology (Mills et al. 2010; De Oliveira et al. 2014). For example, the Laguna Negra orthohantavirus, which causes hantavirus pulmonary syndrome in humans, has been associated with *C. fecundus* in northern Argentina (Pini et al. 2012) and with *C. callosus* s.s. from south-central Bolivia (Carroll et al. 2005), and with *C. callidus* from the state of Mato Grosso in Brazil (Travassos da Rosa et al. 2012). In addition, *Calomys* sp. from the Beni savannah is the reservoir of the Machupo mammarenavirus, the etiological agent

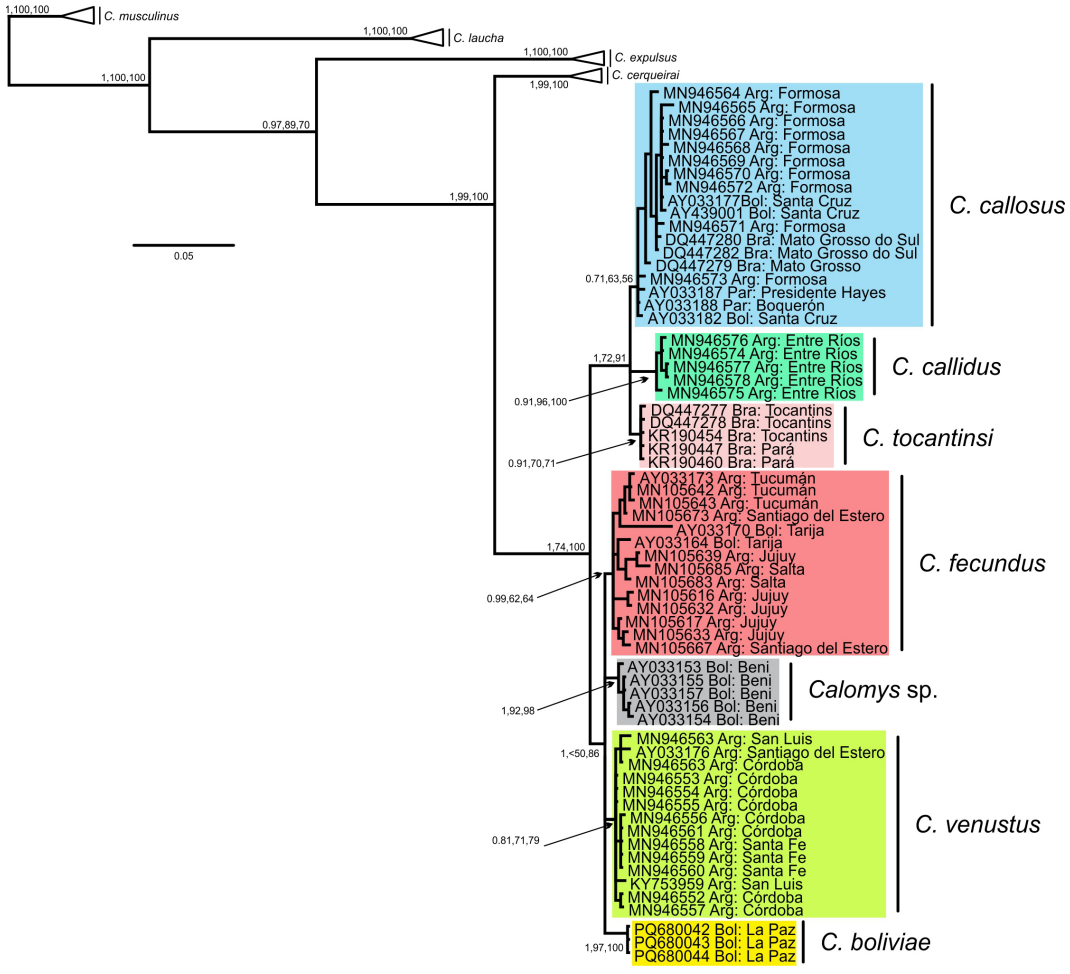

Fig. 3. Phylogram of the Bayesian consensus tree obtained from the Cyt-b dataset of specimens of the *Calomys callosus* complex after 10 million generations using MrBayes 3.2.7. Each clade within the *C. callosus* complex is indicated by a different color and the corresponding species name. Only relevant posterior probabilities are shown. For comparison purposes, sequences of the large-bodied *C. cerqueirai* and *C. expulsus* were included. Sequences of *C. musculinus* and *C. laucha* were used as outgroups.

of the Bolivian hemorrhagic fever (Johnson et al. 1965). In northern Argentina, *C. fecundus* is also a reservoir of a Latino-like mammarenavirus (apparently non-pathogenic; Calderón et al. 2010). *Calomys boliviae*, on the other hand, is one of the least known species of the genus, and in this study, no infected specimens were found near the community of San José. Nevertheless, we have made a contribution by identifying this species for the first time using molecular methods and by demonstrating that it belongs to the *C. callosus* complex. It is clear that this is a great step forward, but we must be aware that in this study we could analyze a few specimens, only from a small area, which does not allow us to evaluate the limits of the real distribution of the species, the genetic diversity, and the geographical structure of its populations, nor whether or not there is sympatry with the other species of the complex. In future studies, it would be important to carry out a more detailed morphological analysis, comparing series, which would allow a modified diagnosis of *C. boliviae* and *C. fecundus*.

Table 3

Matrix of Kimura 2 parameter genetic distances among the species of the *Calomys callosus* complex analyzed in this study. In bold, distances within each clade. In gray, distances among the three species of the eastern lineage. In orange, distances among the four species of the western lineage. In yellow, distances between the species of the eastern and western lineages.

	1	2	3	4	5	6	7
1. *C. callosus* s.s.	**0.48 ± 0.33**						
2. *C. callidus*	1.57 ± 0.22	**0.17 ± 0.09**					
3. *C. tocantinsi*	0.85 ± 0.26	1.14 ± 0.21	**0.06 ± 0.07**				
4. *C. fecundus*	2.42 ± 0.52	2.93 ± 0.42	1.90 ± 0.74	**0.84 ± 0.61**			
5. *C. venustus*	2.23 ± 0.23	2.74 ± 0.15	1.80 ± 0.43	1.16 ± 0.48	**0.23 ± 0.18**		
6. *Calomys* sp.	2.46 ± 0.18	3.04 ± 0.09	2.05 ± 0.43	1.22 ± 0.46	1.00 ± 0.14	**0.16 ± 0.10**	
7. *C. boliviae*	2.44 ± 0.14	2.79 ± 0.04	2.15 ± 0.32	1.41 ± 0.40	1.31 ± 0.13	1.34 ± 0.08	**0**

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